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**Abstract**—Extended volume imaging requires the ability to combine volumes in three dimensions. Given a series of layers, volumes imaged sequentially with an overlap in Z, a way is needed to stitch or couple the layers together to create a single volume. This poster describes an automated script that uses the feature-detecting algorithm Speeded Up Robust Features to identify and match slices in the overlap between volume layers. Once all the layers have been matched, the script saves a new extended volume for viewing, allowing an overall structure of a sample to be analyzed.

**Index Terms**—SURF, confocal, feature detection, volume creation

## I. Introduction

Laser scanning confocal microscopy provides sub-micron resolution volumetric data. A small field of view in the X and Y can be overcome using tiled imaging and stitching together laterally adjacent volumes. To extend the field of view in Z, imaging techniques such as Vibratome Assisted SubSurface Imaging Microscopy (Vibra-SSIM) generate layers of confocal volumes that overlap in Z with the next underlying layer [2]. To stitch together these confocal extended Z volumes with their unique caveats, the applicability of XY and 3D stitching techniques were evaluated. A coupling based on feature detection offered good results for creating extended volumes.

The feature detector Speeded Up Robust Features (SURF)

was chosen. SURF uses an approximation of the determinant of a Hessian to locate features in an image. The second order derivatives of Gaussians of differing scales used to determine the Hessian are approximated using box filters. Features are described using Haar wavelet responses centered on a feature interest point [1],[3].

## II. Procedure

The automated script was written in MATLAB using functions from the Computer Vision System Toolbox, including SURF and a geometric transform estimation, MLESAC [5]. To run properly, the script required two pieces of information: 1) the number of separate confocal volume (layers) used to make the final volume and 2) where the overlap begins according to Z position (slice) in a layer. Here an increase in n or in slice number indicates an increase in the Z position.

- 1) Input: Image sequence containing all layers, number of layers, slice position of overlap.
- 2) Output: Position of best matches for creating extended volume
- 3) Initialize variables
- 4) LOOP
- 5) For number of layer – 1 do
- 6)     Read in slice 1 of layer n+1
- 7)     Detect SURF Features
- 8)     Embedded LOOP
- 9)     For position of overlap +/- search bracket do
- 10)         Read in current search slice of layer n
- 11)         Detect SURF Features
- 12)         Compare features by estimating geometric transforms
- 13)         Store number of matches
- 14)     End
- 15)     Find highest number of matches
- 16)     Record layer n slice number with highest matches
- 17) End

Once all the layers have been compared, and the best matches found, a new volume is saved with a canvas size increase. The Fiji plugin StackReg is used to register the volume [4].

Currently, the volume is inspected as a quality control and manually corrected if errors are found. Errors appear as lines running across the volume when viewed orthogonally on the XZ or YZ plane. Errors also can appear as stuttering, hesitating or jumping if viewing the stack as a movie moving through Z.

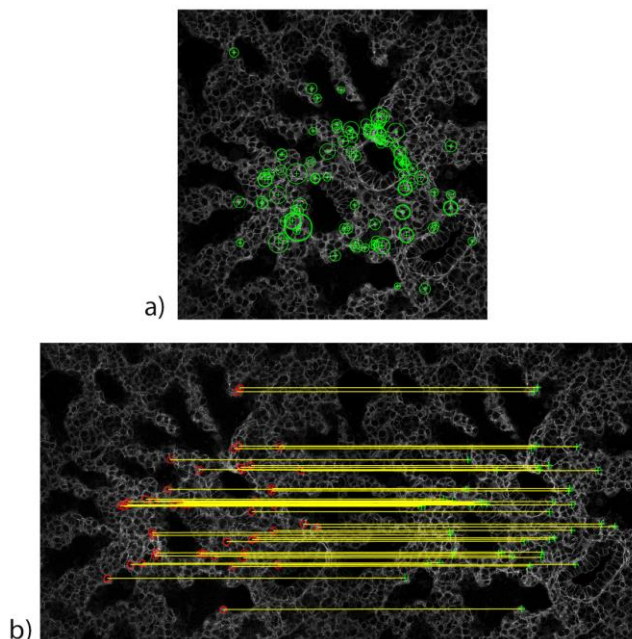


Fig. 1. Taken from the script matching embryonic mouse lung, a) the green circles represent the first 100 of 3676 SURF features found for layer 02, slice 00, b) the first 50 of 371 matches found between layer 02, slice 00 on the right and layer 01, slice 52 on the left. This was found as the best match.

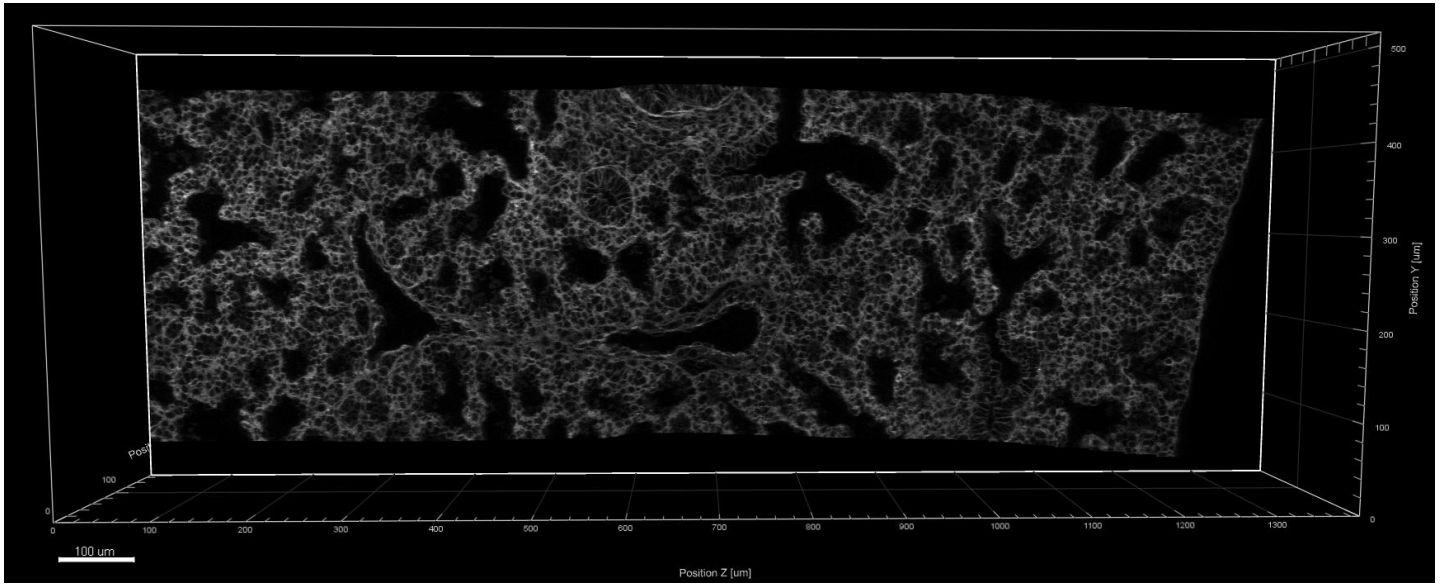


Fig. 2. Full volume, 33 layers coupled in Z and registered in X and Y, viewed as an orthogonal slice along the YZ axis in Imaris. The scale bar is 100  $\mu\text{m}$ . Note the black edges around the data are the canvas padding added before registration.

Visualization in either Imaris or Fiji provided the quickest and easiest way to search for errors.

### III. Experimental Results

The script was run on a data set #7760A, an E18.5 ROSA26:mG/mT mouse lung where all of the cells express a membrane localized red fluorescent protein tdTomato. There were 33 individual layers scanned using Vibra-SSIM that represent  $425 \mu\text{m} \times 425 \mu\text{m} \times 60 \mu\text{m}$  volume each with a voxel size of  $0.415 \mu\text{m} \times 0.415 \mu\text{m} \times 0.8 \mu\text{m}$ . An approximate overlap of  $20 \mu\text{m}$  exists between layers. The final coupled extended volume was  $425 \mu\text{m} \times 425 \mu\text{m} \times 1389 \mu\text{m}$ . (Fig. 2)

For this data set, with 33 layers and a 13 total slices searched and compared at each junction, this script took 1341 seconds running on a computer that has an Intel i7-4930k CPU at 3.4GHz and 64.0 GB of RAM.

Using this same data, a series of deformation tests were run. Keeping layer  $n$  in its original orientation, following layers were translated in  $x$  and  $y$ , rotated about the middle and scaled in  $x$  and  $y$  separately and in combination. There were no changes in where the script found the best match between the layers of this volume.

### IV. Discussion

With a few data sets the script has seen satisfactory results. The resultant extended confocal volumes have allowed for detailed segmentation of several biological structures including bronchial airways, pre-alveolar saccules and small vascular branches (fig. 3).

The incoming volume data does suffer limitations from the collection process at this point. Subtle tissue deformations induced by the Vibra-SSIM sectioning process around tissue inhomogeneities can appear as errors in the registration. However, the script relies on enough features that this has not caused a major issue in the coupling though it affects the

quality assessment. This does mean a minimum number of features is required in order for the script to have successful results.

In confocal data there is also uneven brightness and increased scattering in Z, resulting in fewer SURF features and matches. In data that has increased scattering or noise, a best match is usually found earlier than is accurate. The earlier slice retains more features that are close enough to slice one of the next layer that it is called best match.

The script itself has limitations because of its nature. It only has the ability to search in two dimensions and couples layers together at the voxel level. The low resolution along the Z-axis and minor offsets between layers means that the position of

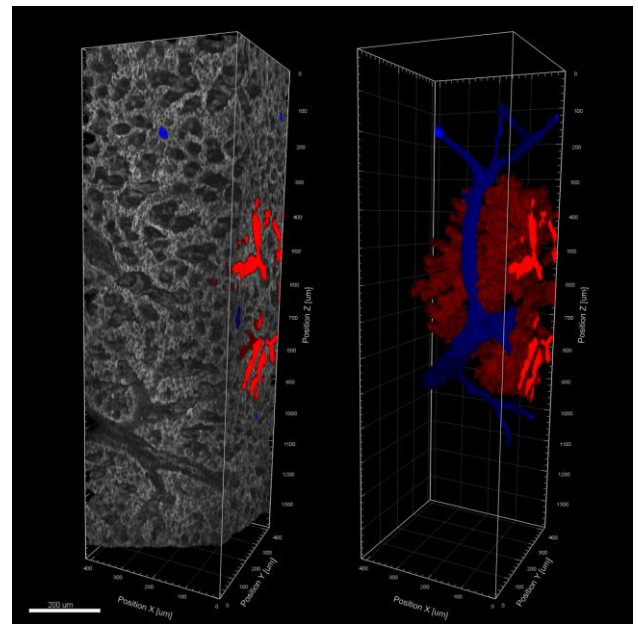


Fig. 3. The same volume rendered in Imaris with two segmented structures, an airway in red and vasculature in blue. The scale bar is  $200 \mu\text{m}$ .

layers at voxel resolution may not be correct.

The script also fails when introducing tiled data. A two by two tiled volume demonstrated that current imaging introduces a tilt between two and three  $\mu\text{m}$  in  $z$  between layers. The procedure detects this offset but currently cannot accommodate it, calling the best match incorrectly and showing distinct lines and errors across the extended volume.

Work will continue to address these specific needs for data along the  $Z$ -axis, to optimize this stitching technique further.

#### V. Acknowledgment

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#### References

- [1] H. Bay, A. Ess, T. Tuytelaars, and L. Van Gool, "SURF: Speeded up robust features", *Computer Vision and Image Understanding* (CVIU), Vol. 110, No. 3, pp. 346-359, 2008
- [2] D. S. Koos, et al. "Extended volume imaging in the lung", *Am J Respir Crit Care Med*, Vol. 191, A2339, 2015
- [3] D. Sargent, C.-I. Chen, C.-M. Tsai, Y.-F. Wang and D. Koppel, "Feature detector and descriptor for medical images," *Proc. of the SPIE medical Imaging Conference*, 2009
- [4] P. Thévenaz, U.E. Rüttimann, and M. Unser, "A pyramid approach to subpixel registration based on intensity", *IEEE Transactions on Image Processing*, vol. & no. 1, pp. 27-41, January 1998
- [5] P.H.S. Torr and A. Zisserman, "MLESAC: A new robust estimator with application to estimating image geometry," *Computer Vision and Image Understanding*, 2000